Pathogen burden in NSW winter cereal cropping

Andrew Milgate and Steven Simpfendorfer, NSW DPI

Key words

disease survey, rotation, PREDICTA® B

GRDC code

DAN00213: Grains Agronomy & Pathology Partnership - *A strategic partnership between GRDC and NSWDPI.* Projects BLG207 and BLG208

Take home message

- Disease surveys are important to stay abreast of developing issues within farming systems
- Fusarium crown rot was widespread in cereal crops in 2019
- The yellow spot fungus was detected in 52% wheat crops surveyed and surprisingly also in 53% of the barley crops.
- qPCR assays (PREDICTA® B platform) are a valuable tool to rapidly quantify a wide range of fungal pathogens, nematode pests and beneficial fungi within wheat and barley crops
- Restoring rotations to include breaks in winter cereal are necessary to reduce pathogen burdens within paddocks.

Introduction

Reducing disease burden in winter cereal crops begins with measurement of what pathogens are present and their severity. This allows for decisions to be made about the need for rotation with alternative crop species and or integration of other control strategies.

The lack of good seasonal planting conditions has put pressure on rotation choices for growers across NSW in recent years. Prolonged periods of drought not only affect yield in a particular season but can also impact on the survival and build-up of many pathogens across seasons. This creates greater risk for losses in subsequent years and may take several seasons to return pathogen populations to acceptable levels.

Healthy cropping systems rely on using alternate hosts in a cropping sequence to reduce the frequency a pathogen encounters susceptible material and keeps population levels below economic thresholds for control. This approach is very old and forms the basis of sustainable farming practices around the globe by reducing the need for fungicides and other agrochemical inputs. However, in some regions there is limited alternative crops to plant in rotation with winter cereals. This may be because of a lack of agronomic fitness such as chilling tolerance in chickpeas, perceived lack of marketing options for some pulse crops or high establishment costs for crops such as canola. The most common rotations involve at least two consecutive cereals either as wheat - wheat or wheat – barley sequences. This is increasing the disease burden in our region.

The widespread adoption of stubble retention for improving moisture capture and retention has also led to increased impact from pathogens that survive from one crop to the next on stubble. These stubble-borne pathogens survive on dead plant material and form fruiting structures which produce spores or mycelium to infect the next crop. Examples of these are including *F. pseudograminearum* (cause of crown rot), *Rhizoctonia solani* (cause of Rhizoctonia bare patch) and *Pyrenophora tritici repentis* (cause of yellow leaf spot).

This paper will discuss the latest survey results for four key winter cereal pathogens across the northern region.

What we did

In 2019 the NSW DPI undertook an extensive survey of winter cereal crops in the northern region to determine current disease levels across the region as part of the Grains Agronomy Pathology Partnership. In collaboration with locally based agronomists, 264 winter cereal crops were surveyed between the start and end of grain filling. The GPS location and background information for each paddock were recorded, but to maintain confidentiality, data is presented here based on broad boundaries and distribution maps (Table 1 and Figure 1). Northern NSW sites were located at latitudes north of Tamworth, central NSW sites were situated at latitudes between Tamworth and West Wyalong, and southern NSW sites were latitudes south of West Wyalong. East and west locations were defined by the Newell Highway. The Liverpool Plains is included with NE NSW in the tables with Sth QLD and NW NSW grouped together. Durum and bread wheat data were also combined for simplicity in the tables. In 2019, survey sites and numbers were affected by crop availability in a drought affected season, particularly in central and northern NSW.

Table 1. The regional sampling of winter cereal crops in the northern region for 2019

Region	Barley	Durum	Wheat	Total
CE NSW	8		17	25
CW NSW	22	1	52	75
N Coast	3		4	7
NE NSW	24	9	34	67
NW NSW	2		20	22
SE NSW	13		26	39
Sth QLD	6		12	18
SW NSW	4		7	11
Total	***82***	***10***	***172***	***264***

Within each crop, a diagonal transect (~500 m) was created starting at least 50 m in from a road or fence line and avoiding obvious barriers such as trees or dams. Five consecutive whole plants (roots with adhering soil, stems and heads) were collected along the planting row from ten separate sampling points across the diagonal transect (i.e. total of 50 plants/crop). Samples were transported to Tamworth or Wagga Wagga and stored at 4°C before processing;

- 100 random tillers (i.e. two/plant) were assessed for incidence of basal browning (crown rot), leaf diseases (e.g. yellow spot or net blotch) and head infections (e.g. bunt, smut or Fusarium head blight (FHB)
- Fifty crown and stem bases (one/plant) were rated for the severity of basal browning and scored for root health prior to plating on laboratory media to determine the incidence of *Fusarium* (crown rot) and *Bipolaris sorokiniaina* (common root rot) infection.

The 100 tillers used for visual assessments were further separated into plant base (root, crowns and stems and leaves below the base of the flag-2 leaf) and plant top (stem, leaves above and including the flag-2 leaf and heads) samples. The aim was to determine pathogen loads in the lower section of the cereal plants in comparison to levels in the top three leaves (plus head) which are the main yield contributing leaves in cereal plants. It is expected that leaf pathogen levels in the top three leaves will have a stronger relationship with yield loss from leaf diseases (e.g. yellow spot and net blotches). All samples were dried at 40°C for 48h, put through a fine grinder and then couriered to SARDI to

assess fungal DNA concentrations using a range of existing qPCR assays (available through PREDICTA B). A 20g subsample of ground plant material was mixed with 180g of sterile sand before extraction of total DNA and qPCR B analysis. All DNA data, picograms (pg) or 1000 DNA copies (kDNA) were then converted to 'per gram of dry plant tissue weight' units.

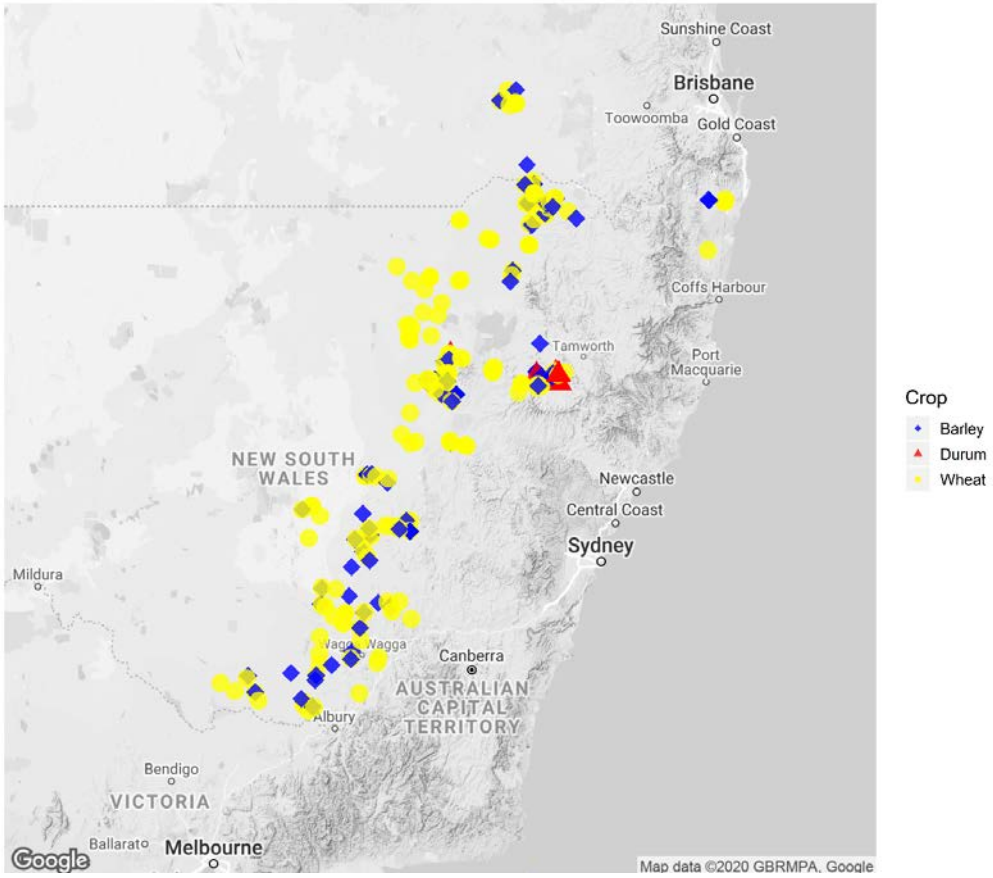

Figure 1. Distribution of winter cereal survey sites across the northern region in 2019

What did we find?

It is important to understand qPCR DNA assays are extremely sensitive with specificity to the target fungal pathogen or plant parasitic nematode of interest. Hence, we expect there to be high levels of pathogen found when infections occur. The approach used in this survey differs from traditional PREDICTA B soil testing where calibrations have been developed to determine the relative risk of infection prior to sowing. Traditional PREDICTA B tests quantify the amount of target pathogen DNA in the soil, old plant roots and stubble residues. This approach helps define the risk of infection developing within a season.

In this survey, plant samples were collected during grain filling and washed to remove soil and old stubble residues. Hence, the DNA tests in this context, determine the level of pathogen burden within either the base or top of the plant at a specified growth stage and do not measure contamination from previous crop residues.

The key point being the DNA values presented in the following tables and figures should ***not*** be compared with current PREDICTA B pre-sowing risk levels or population densities for the different pathogens. Furthermore, the DNA values within plant tops or bases have been assigned to a purely arbitrary low, medium or high category based on the spread of data across sites in 2018 and 2019. They should ***not*** necessarily be interpreted as low, medium or high infection levels.

For example, the 2018 and 2019 seasons were generally dry which was not conducive to the development of leaf diseases such as yellow spot in wheat and net blotches in barley. Hence, even though DNA of the causal fungal pathogens was detected in both seasons, these levels are probably considerably lower than what is likely to be detected in a wetter year. However, DNA concentrations did correlate with visual assessments of disease incidence e.g. crops with higher incidence and severity of basal browning had elevated *Fusarium* DNA levels. DNA data at this stage should be considered for comparative purposes only with continued surveys and research, hopefully developing relationships between pathogen burden in plant bases and/or tops with disease severity and yield loss. Enough of the caveats. What was actually interesting using this new approach with PREDICTA B qPCR assays?

Fusarium crown rot (Fusarium spp.)

Two DNA assays were used to detect *Fusarium pseudograminearum,* with separate tests detecting *F. culmorum* or *F. graminearum*. All three *Fusarium* species can cause basal infection of cereal stems resulting in crown rot and the expression of whiteheads when heat and/or moisture stress occurs during grain fill. When wet weather occurs during flowering, these species, especially *F. graminearum* and *F. culmorum* can also infect heads causing Fusarium head blight (FHB). Due to dry conditions, FHB was not visually recorded in 2019. DNA data for all four tests were combined for this interpretation to provide an overall level of *Fusarium* spp. DNA.

The incidence of crown rot, based on basal browning and laboratory plating, was high across much of the surveyed area in 2019 (data not shown) which reflects the qPCR data (Table 2 and Figure 2). The dry and hot finish to the 2019 season was conducive to the development of Fusarium crown rot, with *Fusarium* DNA detected in the bases of all 264 cereal crops randomly surveyed in 2019 (Table 2, Figure 2). Fusarium crown rot appeared to be particularly severe in cereal crops in central-east, central-west and south-western NSW with 80%, 68% and 64% of wheat and barley crops respectively having high levels of *Fusarium* DNA in the base of plants (Table 2). Fusarium crown rot appears to have also become a significant issue in central and southern NSW especially compared with northern NSW where this disease has traditionally been considered to be more prevalent.

As noted previously, *F. pseudograminearum* is primarily considered a crown and lower stem pathogen, but interestingly low levels of *Fusarium* DNA were also detected in the top section of 65% of samples across regions, indicating the extent of vertical fungal growth up infected tillers under conducive conditions in 2019. In some crops the DNA levels were in the medium to high range in the tops of infected plants (Table 2, Figure 2).

Table 2. Proportion of paddocks (%) with varying levels of *Fusarium* spp. (crown rot) DNA detected in wheat and barley bases or tops in 2019

Fusarium spp. (pg DNA/g)	Bases				Tops			
Region (no. paddocks)	Nil	Low (<1000)	Medium (<10000)	High (>10000)	Nil	Low (<1000)	Medium (<10000)	High (>10000)
Sth QLD/NW NSW (40)	0	72	15	13	33	64	0	3
NE NSW (67)	0	36	21	43	36	57	6	1
N Coast (7)	0	71	29	0	43	43	14	0
CW NSW (75)	0	24	8	68	11	73	13	3
CE NSW (25)	0	12	8	80	20	80	0	0
SW NSW (11)	0	18	18	64	40	60	0	0
SE NSW (39)	0	44	15	41	38	62	0	0
Total (264)	***0***	***38***	***14***	***48***	***27***	***65***	***6***	***2***

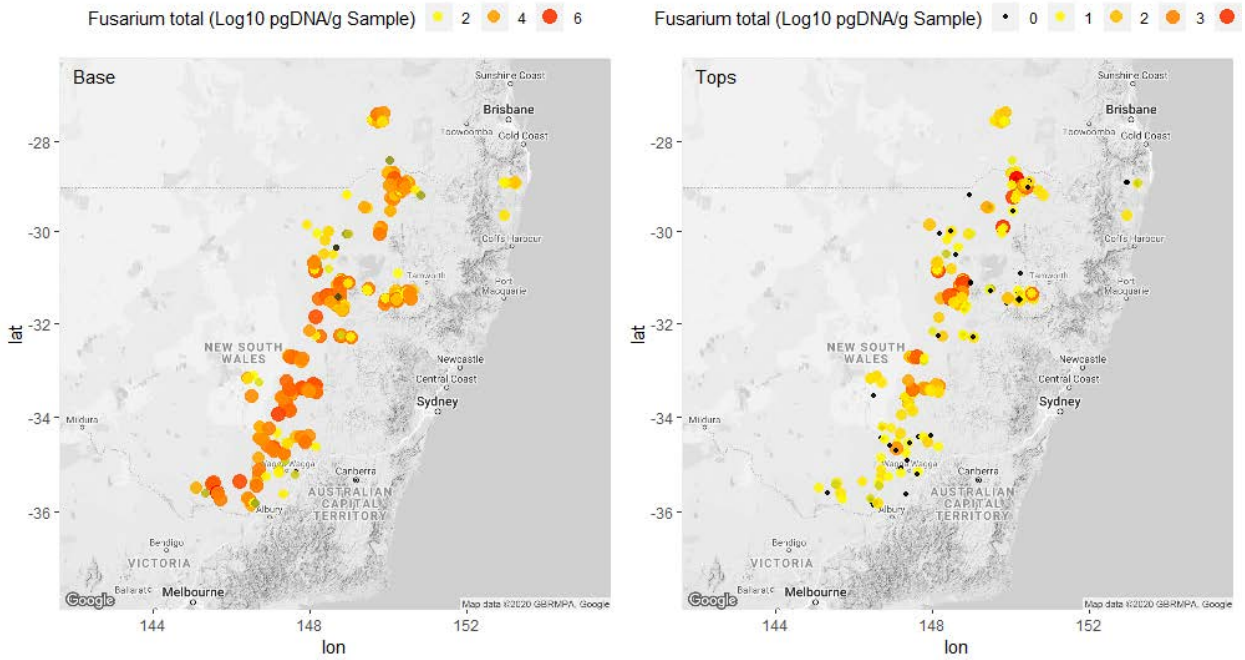

Figure 2. Distribution and intensity of Fusarium crown rot in winter cereal crops across the northern grains region in 2019. 264 paddocks sampled and qPCR used to quantify the presence of Fusarium spp. in the base of wheat and barley plants below the Flag-2 leaf (base; left) and in the plant parts from the Flag-2 leaf up including the heads (tops; right).

Yellow spot (*Pyrenophora tritici-repentis*)

Yellow spot is a stubble-borne disease of durum and bread wheat caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*). Wet weather favours infection and production of tan lesions with a yellow margin on the leaves of susceptible wheat varieties. Repeated rainfall events during the season are required for infection to progress up the canopy of a wheat plant. Given the generally dry conditions in 2019, the visual incidence of yellow spot lesions on the top three leaves during grain filling was low. However, in many crops the presence of yellow spot lesions on the lower leaves was noted (data not shown). Although *Ptr* is a leaf pathogen of wheat, it is also an effective saprophyte (feeds

on dead tissue) and can colonise dead leaves and stubble of barley late in the season under wet conditions as observed in northern NSW in the 2018 survey. Hence, both wheat and barley base and top samples were assayed for *Ptr* DNA levels.

Ptr DNA was detected in the bases of 52% of wheat crops and surprisingly also in 53% of barley crops surveyed in 2019 (Table 3, Figure 3). Medium to high *Ptr* DNA levels were measured in the base of wheat crops across all regions with the exception of north-west NSW and southern Qld. High *Ptr* levels were especially evident in wheat crops in central-east and south-eastern NSW (Table 3, Figure 3).

The proportion of barley crops with medium to high *Ptr* DNA in plant bases was lower compared to wheat and only occurred in central-east, south-eastern NSW and north Coast (Table 3). Underlying differences in rotation sequences or rainfall patterns in 2019 between regions may explain this situation, but this has not yet been explored. Consistent with the 2018 survey, the results for 2019 highlight that barley should not necessarily be considered a break crop for yellow leaf spot in wheat. Recent publications suggest *Ptr* is capable of infecting live barley without causing significant disease symptoms. However, the lifecycle of *Ptr* may also allow it to grow as a saprophyte on dead barley tissue. Further investigation is needed on this issue.

Table 3. Proportion of paddocks (%) with varying levels of *Pyrenophora tritici-repentis* (yellow spot) DNA detected in wheat and barley bases in 2019

Yellow spot (kDNA/g)	Wheat				Barley			
Region (no. paddocks wheat, barley)	Nil	Low (<1000)	Medium (<10000)	High (>10000)	Nil	Low (<1000)	Medium (<10000)	High (>10000)
Sth Qld/NW NSW (29, 8)	69	31	0	0	75	25	0	0
NE NSW (38, 20)	63	29	5	3	75	25	0	0
N Coast (4, 3)	0	50	0	50	0	33	67	0
CW NSW (52, 22)	63	31	6	0	45	55	0	0
CE NSW (16, 8)	6	63	19	13	25	50	25	0
SW NSW (7, 4)	29	29	43	0	25	75	0	0
SE NSW (26, 13)	4	35	42	19	8	62	15	15
Total (172, 78)	***48***	***34***	***13***	***5***	***47***	***45***	***5***	***3***

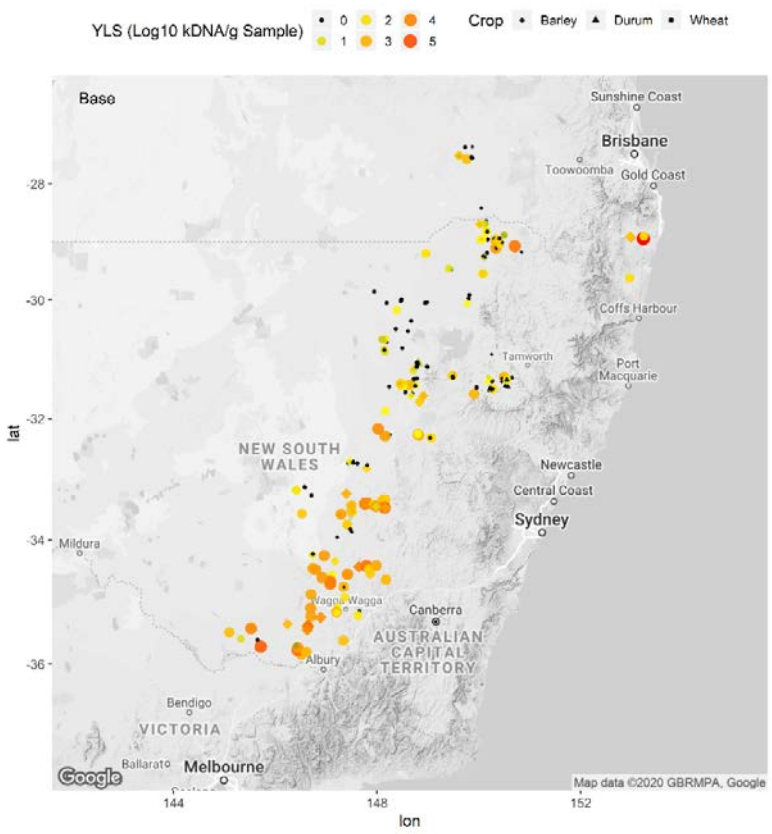

Figure 3. Distribution and intensity of yellow spot in winter cereal crops across the northern grains region in 2019. 264 paddocks sampled and qPCR used to quantify the presence of *Pyrenophora tritici-repentis* in the base of plant below the flag-2 leaf.

Rhizoctonia and Pythium

Rhizoctonia solani (AG8) DNA was only detected on average in 5% of surveyed crops across the northern region in 2019 (Table 4). Prevalence was higher in southern NSW (18% to 25%) than in northern NSW (0%). This is consistent with the known distribution of *Rhizoctonia solani* which is not adapted to survival in the heavier self-mulching alkaline soils which predominate in northern NSW.

Pythium clade f DNA, a pathogen usually associated with seedling blight in wet soils, was detected at low levels in plant bases in 39% of cereal crops surveyed in 2019 (Table 4). Higher detection occurred in southern NSW, followed by central NSW and the north coast with lowest detection in northern NSW in 2019. Medium *Pythium* DNA levels were recorded in 9% of crops in south-west NSW and 8% in south-east NSW in 2019 (Table 4).

Table 4. Proportion of paddocks (%) with varying levels of *Pythium* or *Rhizoctonia solani* AG8 DNA detected in wheat and barley bases in 2019

	Rhizoctonia solani AG8 (*Pg DNA/g)*				*Pythium (Pg DNA/g)*			
Region (no. paddocks)	Nil	Low (<1000)	Medium (<10000)	High (>10000)	Nil	Low (<1000)	Medium (<10000)	High (>10000)
Sth QLD/NW NSW (40)	100	0	0	0	97	3	0	0
NE NSW (67)	100	0	0	0	84	16	0	0
N Coast (7)	100	0	0	0	57	43	0	0
CW NSW (75)	99	1	0	0	52	47	1	0
CE NSW (25)	96	0	4	0	52	48	0	0
SW NSW (11)	82	9	9	0	27	64	9	0
SE NSW (39)	74	10	15	0	21	72	8	0
Total (264)	***95***	***2***	***3***	***0***	***61***	***37***	***2***	***0***

Conclusion

Molecular testing, such as PREDICTA® B qPCR assays used in this survey are a powerful tool for quantifying levels of fungal pathogens, nematode pests or beneficial fungi (AMF) within crop tissue. Dividing plant samples into base (root, crown, stem, leaf below flag-2) and tops (stem, heads and leaves above flag-2) prior to DNA testing, allows an additional level of interpretation. At present DNA concentrations within base or top tissue can only be used for comparative purposes between regions, crops, seasons, rotation sequences, climatic conditions etc. Continuing surveys and associated research are required to understand what impact different DNA concentrations within base or top tissue has on crop yield.

Collectively these survey results show that southern NSW faces a different complex of pathogens compared to northern NSW. Measuring the pathogen burden as this survey has done, highlights the importance for growers in southern NSW to review current rotations and strongly consider including the use of single or double break crops to reduce pathogen burden over time.

Acknowledgements

Research and communication undertaken as part of this project is made possible by the significant contributions of growers through trial cooperation and support of the GRDC. The author would like to thank all for their continued support. The author would also like to acknowledge the ongoing support for northern pathology capacity by NSW DPI. NSW DPI technical assistance from Robyn Shapland, Pat Mortell, Michael McCaig, Tony Goldthorpe in collecting the structured survey samples and organising access to some paddocks; Tim O'Brien, Finn Fensbo, Jason McCulloch and Chrystal Fensbo in processing plant samples is gratefully acknowledged. The efforts of SARDI staff including Daniele Giblot-Ducray, Herdina, Russell Burns and Nigel Percy in rapidly processing the base and top plant samples using PREDICTA® B qPCR assays is also greatly appreciated. We also thank the following agronomists: Leigh Norton, Jeremy Dawson, James Fleming, Glenn Shepherd, Chris Cooke, David Kelly, Zac Geldof, Drew Penberthy, Tony Lockrey, Brad Coleman, Scott Rogers, Matt Gardner, Graeme Callaghan, Greg Rummery, Adam Delwo, Alice Burley, Ben Matthews, Campbell Wilson, Charlie Vallance, Chris Minehan, Chris Toohey, Craig Warren, David Jarrott, Ed Nixon, Emma Ayliffe, Fred Broughton, Glen Dyson, Josh Parker, Joe Bowen, Justin Tidd, Lincoln Harris, Matt McRae, Michael Harris, Nathan Soulsby, Neil Durning, Nigel Clarke, Peter Lott, Phil Gray, Ricky Brett, Rohan Brill, Sandy Biddulph, Stacey Doolan, Tim Stivens and Will Haines who assisted in organising

paddocks to be included in the 2019 survey. We also greatly appreciate all the growers who let us access their cereal crops for this survey.

Contact details

Andrew Milgate
NSW DPI
Pine Gully Rd
Wagga Wagga, NSW 2650
Ph: 02 6938 1990
Email: andrew.milgate@dpi.nsw.gov.au

Steven Simpfendorfer
NSW DPI
4 Marsden Park Rd
Tamworth, NSW 2340
Mb: 0439 581 672
Email: steven.simpfendorfer@dpi.nsw.gov.au